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Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit

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Abstract

The changes in antioxidant capacity, enzyme activity and decay development in strawberry fruit (*Fragaria x ananassa Duch.*) illuminated with different UV-C dosages were investigated. Three UV-C illumination durations and dosages, 1, 5 and 10 min, (0.43, 2.15 and 4.30 kJ m^{-2}) tested promoted the antioxidant capacity and enzyme activities and significantly reduced the severity of decay during storage at 10 °C compared to the control. UV-C illumination for 5 and 10 min showed the best results for enhancing antioxidant capacity expressed as oxygen radical absorbance capacity (ORAC) values after storage for 15 days among all the treatments. These treatments also enhanced the activities of antioxidant enzymes including glutathione peroxidase (GSH-POD), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (AsA-POD), guaiacol peroxidase (G-POD), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR). The nonenzyme components such as reduced glutathione (GSH) and oxidized glutathione (GSSG) also were increased by UV-C exposure. All UV-C dosages increased the phenolic content of strawberry fruits as well. Total anthocyanin content increased during storage in all treatments. However, UV-C illumination showed little effect on the anthocyanin accumulation. All UV-C dosages retarded the development of decay comparing to control treatment, but 5 and 10 min UV-C illumination gave the best decay inhibition.

Keywords: Strawberries; UV; Antioxidant capacity; Antioxidant enzyme activity; Decay

1. Introduction

In recent years, increasing attention has been paid by consumers to the health and nutritional aspects (vitamins content, mineral elements, antioxidants, etc.) of horticultural products (Scalzo et al., 2005). Fruits and vegetables contain significant levels of biologically active components that impart health benefits beyond basic nutrient (Oomah and Mazza, 2000). High consumption of fruits and vegetables has been associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts (Leong and Shui, 2002).

Strawberries are one of the richest sources of natural antioxidants among fruits (Wang et al., 1996; Wang and Zheng, 2001). In addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic compounds (Heinonen et al., 1998). Strawberry fruits have high scavenging capacity against peroxyl radicals (ROO^*), superoxide radicals (O_2^{*-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^*) and singlet oxygen ($^1O_2$) (Wang and Zheng, 2001). In addition to being free radical scavengers, these antioxidants in strawberries are capable of acting as peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors and synergists (Larson, 1988).

Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices, and postharvest handling and processing (Ayala-Zavala et al., 2004). As antioxidant content is becoming an increasingly important parameter with respect to fruit and vegetable quality, it is of great interest to eval-

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uate changes in antioxidant status during postharvest storage of horticultural crops.

UV-C illumination as a postharvest treatment has proven beneficial to delay postharvest fruit senescence and especially control decay in different fruit and vegetable species (Maharaj et al., 1999; Barka et al., 2000; Erkan et al., 2001; Marquenie et al., 2003; Allende and Artes, 2003; Allende et al., 2006). The exposure to UV-C delays fruit softening which is one of the main factors determining fruit postharvest life (Pan et al., 2004). Barka et al. (2000) found that UV-C decreased the activity of enzymes involved in tomato cell wall degradation and delayed the fruit softening. Reduction of strawberry fruit softening by UV-C application has also been reported (Baka et al., 1999).

However, little information is available on the effect of UV-C illumination on antioxidant system in strawberry fruit. Therefore, the purpose of this study was to determine the changes in the antioxidant capacity and enzyme activity in strawberry fruit illuminated with UV-C at different illumination durations and dosages. The effect of UV-C illumination on decay in strawberry fruit was also examined.

2. Materials and methods

2.1. Chemicals

Ascorbate, chlorogenic acid, β-carotene, histidine, hydrogen peroxide (30%, w/w), hydroxylamine hydrochloride, *N*,*N*-dimethyl-*p*-nitrosoaniline, xanthine, xanthine oxide, ascorbate oxidase, dithiothreitol (DTT), glutathione (oxidized form), glutathione (GSH, reduced form), glutathione reductase, guaiacol, β-nicotinamide adenine dinucleotide (β-NADH, reduced form), β-nicotinamide adenine dinucleotide phosphate (β-NADPH, reduced form), nitro blue tetrazolium (NBT), Chelex 100, and $FeSO_4$ were purchased from Sigma Chemical Co. (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and α-tocopherol, and trichloroacetic acid were purchased from Aldrich (Milwaukee, WI). 2′,2′ Azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA).

2.2. Fruit sample handling and illumination with UV-C

Strawberries (*Fragaria x ananassa* cv. Allstar) used in this study were grown at a farm near Beltsville, Maryland, USA and were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Selected strawberries were randomized and used for the experiments. After sorting, fruit were subjected to three UV-C illumination durations (1, 5 and 10 min) with dosages (0.43, 2.15 and 4.30 kJ m^{-2}) by using UVP lamp (Model UVLMS-38: 3UV EL Series UV Lamp, 8 W, LW/MR/SW, Upland California, USA) equipped with a filter (98-0016-02, Upland, California) to have only one wavelength at 254 nm with an intensity of 3 W m^{-2} at a distance of 15 cm. The intensity of the UV-C lamp was determined with a Blak-Ray J-225 photometer. Fruit were placed on a polystyrene net and illuminated with UV-C from both upper and lower sides.

After UV-C illumination, control and illuminated fruit were placed in containers and covered by lid and then the containers were stored at 10 °C. Eight containers were used for each treatment. Control fruits were handled similarly with the exception of the UV-C illumination. Samples were taken initially and at 5, 10 and 15 days intervals during storage. Samples were stored at −80°C until they were assayed for antioxidant capacity and enzyme activity. The development of decay caused by fungi was also evaluated and after 5, 10, 15 and 20 days and expressed as percent of fruit showing any fungal deterioration.

2.3. UV-C illumination

The UV-C illumination device consisted of two banks of 15 stainless-steel reflectors with unfiltered germicidal emitting lamps (FG15T8-15 W T8 120 V germicidal lamp GRM-0152, Atlanta Light Bulbs Inc., Tucker, Georgia, USA) located 15 cm above and below the radiation vessel. The UV-C intensities of the emitting lamps were determined by using a Blak-ray J-225 photometer (Ultra-Violet products, Inc., San Gabriel, California, USA). Although a small amount of infrared radiation was also emitted, 98.7% of the total emitted light was in the UV-C (220–290 nm, with peak radiation at approximately 254 nm) region. The UV-C lamps, reflectors, and treatment area were enclosed in a wooden box covered with aluminum foil and supported by a metal framework to provide protection for the operators. The different UV-C illumination doses were obtained by altering the duration of the exposure at the fixed distance. Prior to use, the UV lamps were allowed to stabilize by turning them on at least 15 min. Three UV-C illumination durations and dosages were applied to strawberry fruit. These durations were 1, 5 and 10 min. These illumination durations were equal to 0.43 (1 min), 2.15 (5 min) and 4.30 (10 min) kJ m^{-2} on each side of the produce. Non-illuminated strawberries were considered as the control treatment.

2.4. Total anthocyanin and total phenolic content

Strawberry fruit were extracted with 80% acetone containing 0.2% formic acid using a Polytron (Brinkmann Instruments, Inc., Westbury, NY). The homogenized samples from acetone extracts were then centrifuged at 14,000 × *g* for 20 min at 4 °C. The supernatants were transferred to vials, stored at −80 °C, and later used for anthocyanin, total phenolic, enzyme and antioxidant analysis.

Total anthocyanin content in fruit extracts was determined using the pH differential method (Cheng and Breen, 1991). Absorbance was measured in a Shimadzu Spectrophotometer (Shimadzu UV-160) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}]$ with a molar extinction coefficient of cyanidin-3-galactoside. Results were expressed as cyanidan-3-galactoside equivalents on a fresh weight basis, mg kg^{-1}.

Total soluble phenolics in the fruit extracts were determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1977) using gallic acid as a standard. Results were

expressed as gallic acid equivalents on a fresh weight basis, g kg^{-1}.

2.5. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader (Huang et al., 2002). The automated sample preparation was performed using a Precision 2000 instrument. The sample series dilution sequence was programmed and controlled by the precision power software. The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration and the net AUC and were expressed as millimole Trolox equivalents per kilogram of fresh weight.

2.6. Antioxidant enzyme measurements

2.6.1. Glutathione peroxidase (GSH-POD) and glutathione reductase (GR)

Four grams of fruit tissue, fresh weight, were homogenized in 4 mL 0.1 mol L^{-1} Tris–HCl buffer (pH 7.8) containing 2 mmol L^{-1} EDTA-Na, 2 mmol L^{-1} dithiothreitol (DTT). The homogenate was centrifuged at 20,000 × *g* for 30 min at 4 °C, and the supernatant was used for the GSH-POD and GR assays.

GSH-POD activity was determined using the method of Tappel (1978) with a slight modification. The reaction mixture contained 0.1 mol L^{-1} Tris–HCl buffer (pH 8.0), 0.4 mmol L^{-1} EDTA, 1.0 mmol L^{-1} NaN_3, 1.0 mmol L^{-1} H_2O_2, 1.0 mmol L^{-1} glutathione (GSH), 0.15 mmol L^{-1} NADPH, 1 unit of glutathione reductase and 100 μL enzyme extract. The total reaction volume was 1.0 mL. The reaction was started by adding H_2O_2. GSH-POD activity was determined by the rate of NADPH oxidation at 340 nm via a spectrophotometer (Shimadzu UV-160A, Shimadzu Scientific Instruments, Columbia, MD).

GR activity was assayed according to Smith et al. (1988). The activity of GR was determined by monitoring glutathione-dependent oxidation of NADPH at 340 nm. The reaction was started by adding GSSG and the rate of oxidation was calculated using the extinction coefficient of NADPH (0.622 mol L^{-1} m^{-1}).

2.6.2. Superoxide dismutase (SOD)

Fruit tissue (4 g) was pulverized in a cold mortar and pestle with 4 mL K-phosphate buffer (0.1 mol L^{-1}, pH 7.3) containing 1 mmol L^{-1} EDTA, 2 mmol L^{-1} DTT. The homogenate was strained through four layers of miracloth and centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was used for assaying the SOD enzyme activity. Total SOD activity was assayed photochemically (Monk et al., 1987; Thayer, 1990). One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under assay conditions.

2.6.3. Ascorbate peroxidase (AsA-POD) and guaiacol peroxidase (G-POD)

Fruit tissue (4 g) was pulverized in a cold mortar and pestle with 4 mL K-phosphate buffer (0.1 mol L^{-1}, pH 7.3) containing 1 mmol L^{-1} EDTA, 2 mmol L^{-1} DTT. The homogenate was centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was used for the AsA-POD, and G-POD assays.

AsA-POD activity was assayed according to the method of Amako et al. (1994). The reaction was started by adding H_2O_2.

The G-POD assay mixture contained 0.1 mol L^{-1} phosphate buffer (pH 6.1), 4 mmol L^{-1} guaiacol as donor, 3 mmol L^{-1} H_2O_2 as substrate and 1.0 mL crude enzyme extract. The total reaction volume was 3.0 mL. The rate of change in absorbance at 420 nm was measured, and the level of enzyme activity was expressed as the difference in absorbance (OD).

2.6.4. Dehydroascorbate reductase (DHAR)

DHAR activity was assayed by measuring the rate of NADPH oxidation at 340 nm (Shigeoka et al., 1980). The reaction mixture contained 50 mmol L^{-1} potassium phosphate, pH 6.1, 0.2 mmol L^{-1} NADPH, 2.5 mmol L^{-1} dehydroascorbate, 2.5 mmol L^{-1} glutathione, 0.6 unit glutathione reductase (GR; from spinach, EC 1.6.4.2) and 0.1 mL of diluted fruit juice (2 mL juice was diluted with 2 mL 50 mmol L^{-1} potassium phosphate, pH 6.1). The reaction was started by adding dehydroascorbate.

2.6.5. Monodehydroascorbate reductase (MDAR)

MDAR activity was assayed by measuring the rate of NADH oxidation at 340 nm (Nakagawara and Sagisaka, 1984). The reaction mixture contained 50 mmol L^{-1} K-phosphate buffer (pH 7.3), 0.2 mmol L^{-1} NADH, 1.0 mmol L^{-1} ascorbate, 1.0 unit of ascorbate oxidase and 0.1 mL of 50 mM K-phosphate buffer (pH 7.3) diluted fruit juice (2-time dilution) in a total volume of 1.0 mL. The reaction was started by adding ascorbate oxidase (from *Cucurbita*, EC 1.10.3.3).

2.7. Nonenzyme component measurements

2.7.1. Reduced glutathione (GSH) and oxidized glutathione (GSSG)

Triplicate 4 g strawberry fruit samples were homogenized in 8.0 mL ice-cold, degassed 7.57 mmol L^{-1} sodium ascorbate solution with chilled mortar and pestle under N_2 at 0 °C. The homogenate was filtered through four layers of miracloth (Calbiochem, La Jolla, Calif.) and centrifuged at 30,000 × *g* for 15 min at 0 °C. The supernatant was deproteined in glass test tubes by incubating in a water bath at 100 °C for 3 min and then centrifuged at 15,000 × *g* for 15 min at 0 °C. The supernatants were used for the GSH and GSSG assays. GSH and GSSG were assayed using the method described by Castillo and Greppin (1988). GSSG was determined by subtraction of GSH from total glutathione.

2.8. Statistical analysis

Experiments were performed according to a factorial design. Data were analyzed by means of a one-way ANOVA. In the case of a significant *F*-value, the means were compared by the least significant difference (LSD) test at a significance level of 0.05.

3. Results

3.1. Total anthocyanin content

Total anthocyanin content from different UV-C illumination dosages is shown in Fig. 1A. Initial anthocyanin content of the strawberries was 390.8 mg kg^{-1}. Anthocyanin content of the strawberries increased during storage in UV-C illuminated and control fruit. However, little difference in anthocyanin content was observed among various treatments during storage at 10 °C (Fig. 1A).

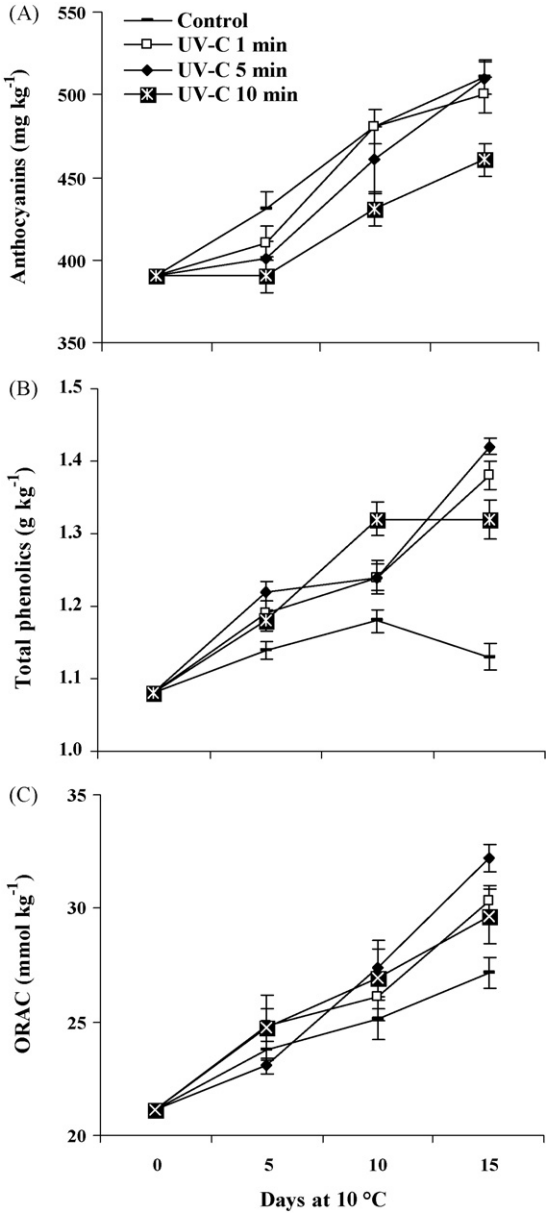

Fig. 1. Total anthocyanins (A), total phenolics (B), and total ORAC (C) values in strawberry fruit illuminated with different UV-C durations. Vertical bars represent standard error.

3.2. Total phenolic content

Total phenolic content from different UV-C illumination dosages is shown in Fig. 1B. Initial phenolic content of strawberries was 1.083 g kg^{-1}. Strawberry fruit illuminated with 5 min UV-C had the highest total phenolic content followed by 1 min and 10 min UV-C illumination. Control fruit had the lowest total phenolic content. Total phenolic content from strawberry fruit for all UV-C illuminations and control treatment increased during 15 days storage period. However, this increase was relatively lower in control fruit when compared to illuminated fruit.

3.3. ORAC values

Antioxidant capacity was expressed as an ORAC values in strawberries (Fig. 1C). The ORAC values of strawberries in all treatments increased during 15 days storage at 10 °C. However, this increase was relatively lower in control fruit when compared to illuminated fruit. Initial ORAC values of strawberries (TE, fresh weight basis) were 21.11 mmol kg^{-1}. Strawberry fruit illuminated with 5 min UV-C and stored for 15 days had the highest total ORAC values (32.21 mmol kg^{-1}) followed by 1 min (30.33 mmol kg^{-1}) and 10 min (29.65 mmol kg^{-1}) UV-C illumination. Control fruit had the lowest total ORAC value which was 27.17 mmol kg^{-1}.

3.4. Antioxidant enzymes

3.4.1. Glutathione peroxidase (GSH-POD) and glutathione reductase (GR)

GSH-POD and GR activities of strawberry extract stored for 15 days were varied among treatments and storage durations as shown in Fig. 2A and B. After 15 days of storage, strawberry extract from 10 min UV-C illuminated treatment had the highest activities. Strawberry extracts from control and 1 min UV-C illumination had lower activities compared with those from 5 and 10 min UV-C illumination. After 15 days storage, samples from the control treatment had the lowest activities for GR and 1 min UV-C illumination had the lowest activities for GSH-POD.

3.4.2. Superoxide dismutase (SOD)

Strawberry extracts taken after 5 days of storage had higher SOD activities than those taken after 10 and 15 days of storage as shown in Fig. 3A. SOD activity of strawberry remained steady during the first 5 days of storage and then decreased during the rest of storage at 10 °C. After 15 days storage, strawberry extracts from all UV treated fruit had higher SOD activities than those from control fruit.

3.4.3. Ascorbate peroxidase (AsA-POD) and guaiacol peroxidase (G-POD)

AsA-POD and G-POD activities of strawberry extract illuminated with UV-C increased initially then decreased during

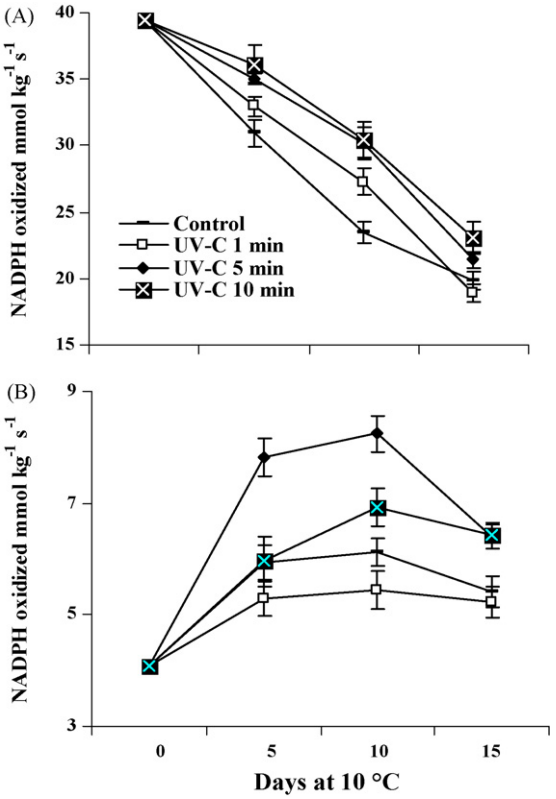

Fig. 2. Glutathione peroxidase (GSH-POD) (A) and glutathione reductase (GR) (B) activities in strawberry fruit illuminated with different UV-C durations. Vertical bars represent standard error.

storage at 10 °C (Fig. 3B and C). After 15 days of storage, while 10 min UV-C illumination had the highest AsA-POD activities in strawberry extract, 5 min UV-C illumination had the highest G-POD activities. While strawberry extract from 1 min UV-C illumination had the lowest activities for AsA-POD, control treatment had the lowest G-POD activities.

3.4.4. Monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR)

MDAR and DHAR activities for strawberry extract varied among treatments. MDAR increased while DHAR decreased during storage at 10 °C (Fig. 4A and B). After 15 days of storage, while 10 min UV-C illumination had the highest MDAR activities in strawberry extract, 5 min UV-C illumination had the highest DHAR activities. Strawberry extract from control fruits had the lowest activities for both MDAR and DHAR.

3.5. Nonenzyme components

3.5.1. Reduced glutathione (GSH) and oxidized glutathione (GSSG)

The amounts of GSH varied from 37.17 to 26.56 μmol kg^{-1} fresh berries and GSSG ranged from 21.73 to 14.71 μmol kg^{-1} fresh weights as shown in Fig. 5A and B. Initial GSH and GSSG activities of strawberries were 26.56 and 14.71 μmol kg^{-1} fresh weights, respectively. During the 15 days of storage at 10 °C, GSSG and GSH activities of strawberry extracts increased and

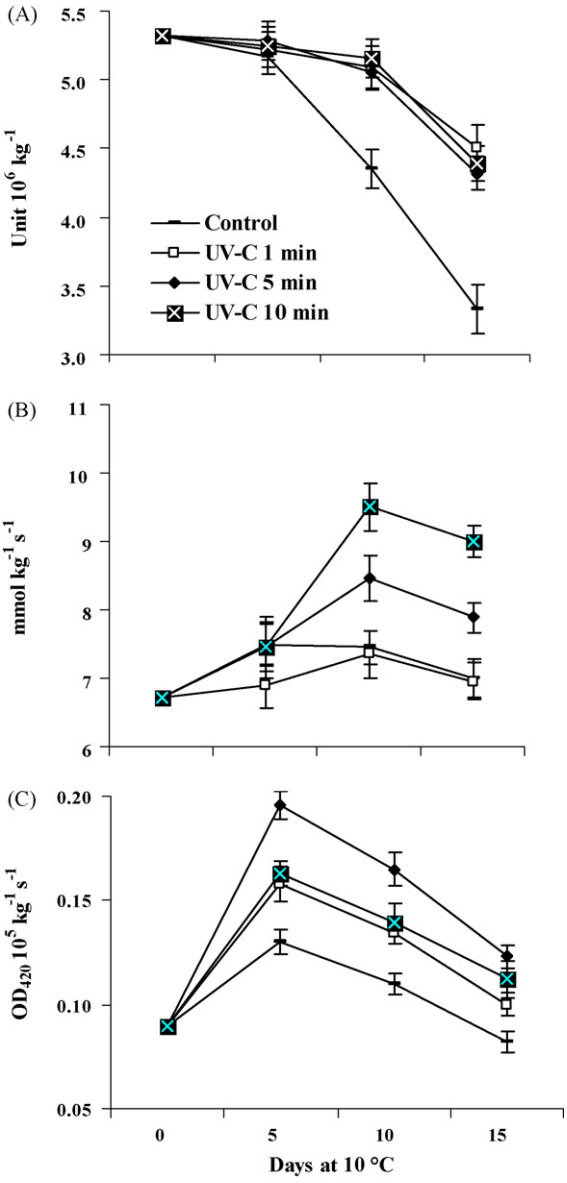

Fig. 3. Superoxide dismutase (SOD) (A), ascorbate peroxidase (AsA-POD) (B), and guaicol peroxidase (G-POD) (C) activities in strawberry fruit illuminated with different UV-C durations. Vertical bars represent standard error.

then decreased. After 15 days of storage, 10 min UV-C illumination had the highest GSSG and GSH activities in strawberry extract (Fig. 5A and B).

3.6. Decay evaluation

Decay of fruit was evaluated for 20 days at 10 °C. All UV-C dosages tested in this study reduced decay in strawberries during storage (Fig. 6). No decay development was observed in all treatments including control fruits during the first 5 days of storage. The most effective treatment controlling decay was illumination of strawberries for 10 min. Percentage of decay was 27.98 after 20 days storage in this treatment. UV-C illumination for 5 and 1 min followed this treatment and decay percentage was 29.6 and 49.64, respectively. Decay develop-

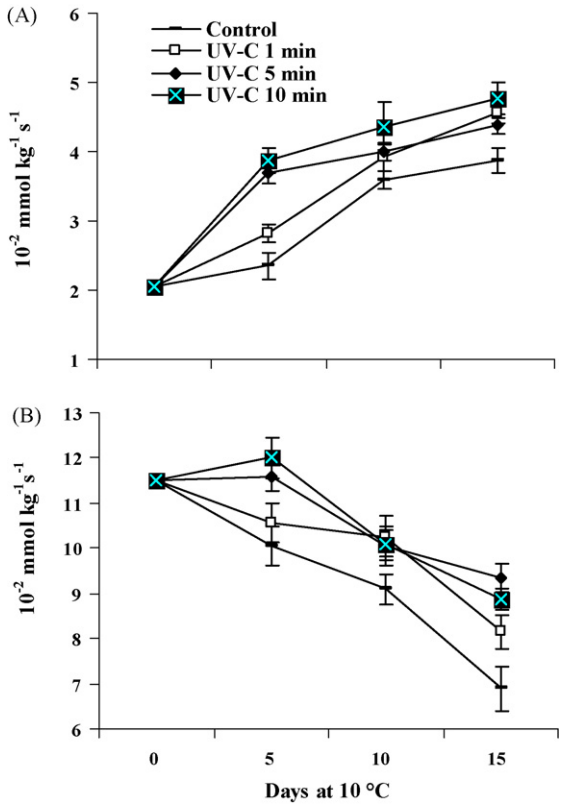

Fig. 4. Monodehydroascorbate reductase (MDAR) (A) and dehydroascorbate reductase (DHAR) (B) activities in strawberry fruit illuminated with different UV-C durations. Vertical bars represent standard error.

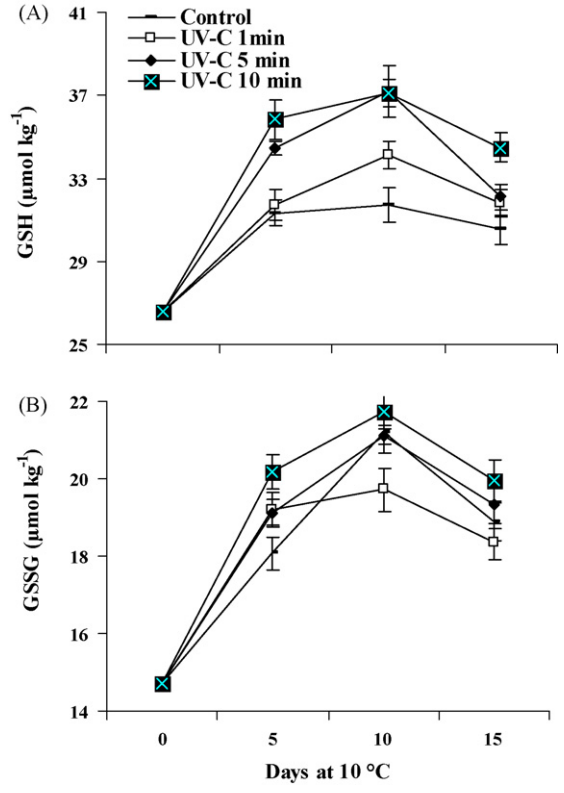

Fig. 5. Reduced glutathione (GSH) (A) and oxidized glutathione (GSSG) (B) activities in strawberry fruit illuminated with different UV-C durations. Vertical bars represent standard error.

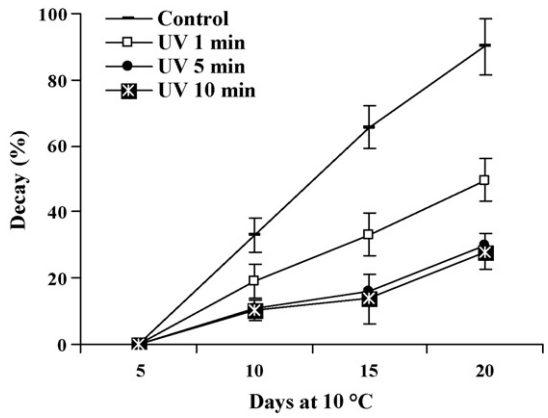

Fig. 6. The effects of various UV-C illumination durations on decay development of strawberry fruit during storage at 10 °C. Vertical bars represent standard error.

ment was the worst in control fruit after 20 days of storage with 89.98%.

4. Discussion

Antioxidants occur naturally in plants. These plant natural antioxidants include phenolic compounds and anthocyanins. Several previous studies have shown that strawberries are also a good source of natural antioxidants (Wang et al., 1996; Wang and Lin, 2000; Wang and Zheng, 2001). Phenolic compounds in fruits and vegetables may produce the beneficial effects by scavenging free radicals (Chun et al., 2003). Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002). Anthocyanins occur almost universally, and they are largely responsible for the red color of ripe strawberries (Ayala-Zavala et al., 2004). In our study, total phenolic and total anthocyanin content were variable in strawberry extract treated with different UV-C illumination dosages. Strawberries stored at 10 °C for 15 days had a higher total phenolic content than strawberries stored for 5 and 10 days. This increase with the storage duration occurred in all UV-C treatments. In control fruit, phenolic content increased during the first 10 days of storage then decreased after 15 days of storage. Previous studies have shown that the amount of total phenolic content in strawberries depends on the storage temperature and atmospheric compositions (Ayala-Zavala et al., 2004; Cordenunsi et al., 2005; Wang and Zheng, 2001).

The main characteristics related to the quality of ripe strawberry fruit are texture, flavor and anthocyanin content (Cordenunsi et al., 2003). Anthocyanins are a group of phenolic compounds responsible for the red–blue color of many fruits and vegetables, and provide beneficial effects to human health (García-Alonso et al., 2004). The amount of anthocyanin is important for the attractiveness and maturity assessments of strawberries as well. In our study, anthocyanin content in strawberry treated with three UV-C illumination dosages ranged between 390.8 and 513.4 mg kg^{-1}. Anthocyanin content increased in all treatments during storage period. That means the strawberries became darker with storage as ripening progresses.

Our results showed that UV-C illumination had little effect on anthocyanin in strawberries. However, several reports have indicated that UV-C exposures promoted anthocyanin synthesis in other fruits, including apples (Dong et al., 1995), sweet cherries (Kataoka et al., 1996), grapes (Kataoka et al., 2003), and boysenberries (Vicente et al., 2004). On the other hand, delay in accumulation of anthocyanin by UV-C illumination has also been reported in strawberry fruit by Pan et al. (2004).

The antioxidant activity in Trolox equivalents (TE) on a fresh weight basis ranged from 21.11 to 32.21 mmol kg^{-1} in strawberry fruit illuminated with three different UV-C dosages. Strawberry extract from 5 min UV-C illumination had the highest ORAC values compared with other UV-C illumination durations (1 and 10 min) and control treatment. Therefore, the ORAC values were positively correlated with total phenolic content, but not with anthocyanin in this study. Kalt et al. (2003) also found a similar relationship among ORAC, total phenolic content, and anthocyanin in highbush blueberries during ripening and storage. Also, even though antioxidant activity was high at 10 °C, this elevated temperature may not be optimal for maintaining the best quality of strawberry fruit during storage (Galletta and Bringhurst, 1990).

The reduced form of glutathione, GSH, is the major non-protein thiol in most plant species. GSH has an important function in maintaining cellular redox status (Rennenberg, 1980). The primary oxidation product of GSH is its disulfide, GSSG, which can be reduced back to GSH by glutathione reductase (GR) at the expense of NADPH (Ric de Vos et al., 1994). The majority of glutathione in the cells is maintained in the reduced state (Kosower and Kosower, 1978). In our study, UV-C illumination at 2.15 and 4.30 kJ m^{-2} enhanced the increase of GSH in strawberry fruit during storage. This reduced form of glutathione plays an important role in the stabilization of many enzymes. It also serves as a substrate for DHAR and reacts directly with free radicals including hydroxyl radical to prevent the inactivation of enzymes by oxidation of the essential thiol group (Ziegler, 1985).

GR activity first increased during the first 10 days of strawberry fruit then decreased after 15 days of storage. 5 min UV-C illumination had the highest GR activity. GR is an ubiquitous NADPH dependent enzyme. It adds hydrogen ion to the oxidized glutathione to regenerate reduced glutathione. In contrast to GR, GSH-POD activity decreased during the storage period. However, after 15 days of storage, 10 min UV-C illumination had the highest GSH-POD activity. This enzyme glutathione peroxidase utilizes reduced glutathione to eliminate hydrogen peroxide and convert it to harmless water. The activity of GSH-POD is dependent on the availability of the reduced ascorbate and GSH that are maintained by enzymes, such as GR, DHAR, and MDHAR using NADPH as an electron donor (Roxas et al., 2000).

Guaiacol peroxidase (G-POD) and ascorbate peroxidase (AsA-POD) are peroxidase enzymes that are found in animal, plant and microorganism tissues, which can catalyze oxidoreduction between hydrogen peroxide (H_2O_2) and various reductants (Hiraga et al., 2001). AsA-POD, involved in the detoxification of H_2O_2, uses two molecules of ascorbate to reduce H_2O_2 to water (Noctor and Foyer, 1998). While 5 min UV-C illumination showed the highest activities in G-POD (5 days after storage), 10 min UV-C illumination showed the highest activities in AsA-POD (10 days after storage). Control fruits had the lowest activities in G-POD and 1 min UV-C illumination had the lowest activities in AsA-POD. Since the biological function of G-POD and AsA-POD is for removal of H_2O_2 and other hydroperoxides, higher activities of G-POD and AsA-POD in the UV-C treated strawberries would be beneficial for protection against lipid peroxidation and DNA hydroperoxides in the fruit (Chaudiere and Ferrari-Iliou, 1999).

Superoxide dismutases (SOD), a class of metal-containing proteins, catalyze the dismutation reaction of superoxide radical anions into H_2O_2 and molecular oxygen (Scandalios, 1993). SOD removes singlet oxygen, prevents formation of hydroxyl radicals and has been implicated as an essential defense against the potent toxicity of oxygen (Fridovich, 1986; McCord, 1979). There are three different types of SOD according to their metal cofactor: Cu/Zn (Cu/Zn-SOD), Mn (Mn-SOD), and Fe (Fe-SOD) (McKersie et al., 1993). However, in this study we determined only the total SOD activity in strawberries treated with different UV-C illuminations. The results showed that SOD activities decreased in all treatments during storage at 10 °C. However, after 15 days of storage, all UV-C illumination treated strawberry fruit had higher SOD activities than the control fruit.

Control and UV-C illuminated fruit did not show any visible infection during the first 5 days of storage. However, the percentage of the decay reached to 89.98 in control fruits in 20 days at 10 °C. Treatment of 10 min UV-C illumination gave the best results for decay control and after 20 days of storage the percentage of decay in this treatment was only 27.98. This might be a result of direct inhibition of microbial growth by UV-C illumination (Allende et al., 2006). It can also be explained that different types of stress such as UV-C illumination can activate defense responses in fruit and thus contribute to alleviate and reduce tissue colonization by pathogen (Pan et al., 2004). Nigro et al. (2000) and Marquenie et al. (2003) inoculated *Botrytis* spores on strawberries and found a reduction of decay in UV-C illuminated fruit, indicating that fruit defensive responses could be activated. It is also possible that UV-C illumination induces and activates decay-resistance mechanisms by increasing the expression of antifungal genes and compounds in the fruit peel.

In conclusion, this study showed that strawberries illuminated with UV-C consistently had higher antioxidant capacity and enzyme activity and less decay than control fruit. These results suggest that UV-C treatments may be a useful non-chemical way of maintaining strawberry fruit quality and extending their postharvest life. Further investigation is needed to elucidate the underlying relationship between UV-C illumination and antioxidant capacity in strawberries.

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